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U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

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INTERNATIONAL APPLICATION NO.

PCT/EP99/09833

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PRIORITY DATE CLAIMED

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TITLE OF INVENTION

DRUG TARGETS IN CANDIDA ALBICANS

APPLICANT(S) FOR DO/EO/US :

CONTRERAS

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An executed oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ Assignment documents (3) for recording. Separate cover sheets in compliance with 37 CFR 3.28 and 3.31 are included.
13. ☐ A FIRST preliminary amendment.  
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☒ A substitute specification in 71 pages including 30 claims, a sequence listing, and 4 figures.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: Copy of the International Preliminary Examination Report; Copy of the International Search Report; Associate Power of Attorney; Sequence Disk; Sequence Listing, Verified Statement.

JP18 Rec-0 CT/PTO 04 JUN 2001

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER
09/857372		PCT/EP99/09833		JAB-1430
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):				CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1070.00  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....\$930.00  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.455(a)(2)) paid to USPTO..... \$790.00  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$720.00  International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$98.00				
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$ 930.00
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	30 - 20 =	10	x \$18.00	\$180.00
Independent claims	20 - 3 =	17	x \$80.00	\$1360.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$
TOTAL OF ABOVE CALCULATIONS =				\$2470.00
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$
SUBTOTAL =				\$
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$
TOTAL NATIONAL FEE =				\$2470.00
Fee for recording the enclosed 3 assignments (37 CFR 1.21(h)). The assignments must be accompanied by an appropriate cover sheets (37 CFR 3.28, 3.31). \$40.00 per property +				\$120.00
TOTAL FEES ENCLOSED =				\$2470.00
				Amount to be refunded:
				\$
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				\$ 2590.00
a. <input type="checkbox"/> A check in the amount of \$ _____ to cover the above fees is enclosed.  b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>10-0750/JAB-1430/MHM</u> in the amount of \$2270.00 to cover the above fees. A duplicate copy of this sheet is enclosed.  c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>10-0750/JAB-1430/MHM</u> . A duplicate copy of this sheet is enclosed.				
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.				
SEND ALL CORRESPONDENCE TO:  Philip S. Johnson, Esq. Johnson & Johnson One Johnson & Johnson Plaza New Brunswick, NJ 08933-7003 USA				
Signature <u>Myra H. McCormack</u> Myra H. McCormack Reg. No. 36,602 Attorney for Applicants June 4, 2001				

Q/PRTS

- 1 -

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## DRUG TARGETS IN CANDIDA ALBICANS

### Field of the Invention

5 The present invention is concerned with the  
identification of genes or functional fragments  
thereof from *Candida albicans* which are critical for  
growth and cell division and which genes may be used  
as selective drug targets to treat *Candida albicans*  
associated infections. Novel nucleic acid sequences  
10 from *Candida albicans* are also provided and which  
encode the polypeptides, which are critical for growth  
of *Candida albicans*.

### Background of the Invention

15 Opportunistic infections in immunocompromised  
hosts represent an increasingly common cause of  
mortality and morbidity. *Candida* species are among  
the most commonly identified fungal pathogens  
associated with such opportunistic infections, with  
20 *Candida albicans* being the most common species. Such  
fungal infections are thus problematical in, for  
example, AIDS populations in addition to normal  
healthy women where *Candida albicans* yeasts represent  
the most common cause of vulvovaginitis.

25 Although compounds do exist for treating such  
disorders, such as, amphotericin, these drugs are  
generally limited in their treatment because of their  
toxicity and side effects. Therefore, there exists a  
need for new compounds, which may be used to treat  
30 *Candida* associated infections in addition to  
compounds, which are selective in their action against  
*Candida albicans*.

Classical approaches for identifying anti-fungal  
compounds have relied almost exclusively on inhibition  
35 of fungal or yeast growth as an endpoint. Libraries  
of natural products, semi-synthetic, or synthetic

chemicals are screened for their ability to kill or arrest growth of the target pathogen or a related nonpathogenic model organism. These tests are cumbersome and provide no information about a  
5 compounds mechanism of action. The promising lead compounds that emerge from such screens must then be tested for possible host-toxicity and detailed mechanism of action studies must subsequently be conducted to identify the affected molecular target.

10

### Summary of the Invention

The present inventors have now identified a range of nucleic acid sequences from *Candida albicans*, which  
15 encode polypeptides, which are critical for its survival and growth. These sequences represent novel targets which can be incorporated into an assay to selectively identify compounds capable of inhibiting expression of such polypeptides and their potential  
20 use in alleviating diseases or conditions associated with *Candida albicans* infection.

In a first aspect of the invention, the invention relates to a nucleic acid molecule encoding a polypeptide which is critical for survival and growth  
25 of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9. More preferably where nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 3 and still  
30 more preferably consisting of SEQ ID NO:1 and SEQ ID NO:2.

In a preferred embodiment of this aspect of the invention, the nucleic acid molecule is either RNA or DNA, and in one embodiment, cDNA. The invention also  
35 relates to a first nucleic acid molecule capable of hybridising to a second nucleic acid molecule encoding a polypeptide which is critical for survival and

growth of the yeast *Candida albicans* and which second nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 under high stringency conditions. In yet another embodiment, the invention relates to an antisense molecule comprising a first nucleic acid molecule capable of hybridising to a second nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* wherein the second nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

The invention also relates to cells containing a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein said cells are bacterial or eukaryotic. The invention further relates to polypeptide encoded by a nucleic acid molecule which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 and to polypeptide having any of amino acid sequences selected from the group consisting of SEQ ID NO: 10 to SEQ ID NO: 16.

The invention also relates to a recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein the nucleic acid molecule is DNA. Preferably the DNA is an expression vector and preferably the expression vector includes an inducible promoter and/or a reporter molecule. The invention also relates to recombinant DNA constructs comprising a first nucleic acid molecule wherein the first nucleic acid molecule or a molecule

complementary to the first nucleic acid molecule is capable of hybridising to a second nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and  
5 which second nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein the first nucleic acid molecule is inserted in the antisense orientation.

The invention also relates to cells containing a  
10 recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein  
15 said cells are bacterial or eukaryotic.

In another aspect of this invention, the nucleic acid molecules or polypeptides of this invention are provided in a pharmaceutically acceptable carrier, diluent or excipient.

20 The invention further relates to a method for treating a *Candida albicans*-associated disease comprising the step of: administering a composition of matter comprising an antisense nucleic acid molecule capable of binding to a *Candida albicans*-originating  
25 nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9.

In another aspect of this invention, the invention relates to a *Candida albicans* cell comprising an induced mutation in the DNA sequence  
30 encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* wherein the polypeptide is encoded by a nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

35 The invention also relates to a method of identifying compounds which selectively modulate expression or functionality of polypeptides or

metabolic pathways in which these polypeptides are involved and which polypeptides are crucial for growth and survival of *Candida albicans*, which method comprises: contacting a compound to be tested with one  
5 or more *Candida albicans* cells having a mutation in a nucleic acid molecule, the nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group  
10 consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein the mutation results in overexpression or underexpression of said polypeptide in addition to contacting one or more wild type *Candida albicans* cells with said compound; and monitoring the growth and/or activity of  
15 said mutated cell compared to said wild type; wherein differential growth or activity of said one or more mutated *Candida* cells is indicative of selective action of said compound on a polypeptide or another polypeptide in the same or a parallel pathway. The  
20 invention further relates to compounds identified by this method. The compounds can be provided in a pharmaceutical composition with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The invention also relates to a method of  
25 identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival of said cell or organism, which method comprises: preparing a cDNA or genomic library from said cell or organism in a suitable expression  
30 vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library; selecting transformants exhibiting impaired growth and  
35 determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant. In a preferred aspect

of this invention, the cell or organism is a yeast or filamentous fungus and more preferably the cell or organism is any of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

5       The invention further relates to antibody capable of specifically binding to a polypeptide of this invention. The invention also relates to oligonucleotides comprising a fragment of from 10 to 120 contiguous nucleotides of a nucleic acid molecule  
10       encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9. Preferably the oligonucleotide comprises a fragment of  
15       from 10 to 50 contiguous nucleotides.

#### **Brief Description of the Figures**

Figure 1 is a diagrammatic representation of plasmid pGAL1PNiST-1.

20       Figure 2 is a diagrammatic representation of plasmid pGAL1PSiST-1.

Figure 3 is an exemplary growth curve of a *Candida albicans* strain demonstrating antisense-induced reduction in growth in response to  
25       intracellular antisense expressing specifically targeting one of the nucleic acid sequences of this invention.

Figure 4 is a representative growth curve of a *Candida albicans* knock out strain as compared to wild  
30       type *Candida albicans*.

#### **Detailed Description of the Invention**

According to a first aspect of the invention there is provided a nucleic acid molecule encoding a  
35       polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid



molecule comprises any of the sequences of nucleotides illustrated in any of Sequence ID Nos. 1 to 9.

Whilst the molecules defined herein have been established as being critical for growth and metabolism of *Candida albicans*, for some of the molecules no apparent functionality has been assigned by virtue of the fact that no functionally related sequences in other prokaryotic or eukaryotic organism can be found in respective databases. Thus, advantageously these sequences may be species specific in which case they may be used as selective targets for treatment of diseases mediated by *Candida Albicans* infection. Thus, in one aspect of the invention the nucleic acid molecules preferably comprise the sequences identified in sequence ID Nos. 1, 4, 5 to 9.

In another aspect of the invention the sequences have been arranged functionally and of nucleotides illustrated in Sequence ID Nos. 2 or 3 are preferred and even more preferably in Sequence ID No. 2 and fragments or derivatives of said nucleic acid molecules.

Letters utilised in the sequences according to the invention which are not recognisable as letters of the genetic code signify a position in the nucleic acid sequence where one or more of bases A, G, C or T can occupy the nucleotide position. Representative letters used to identify the range of bases which can be used are as follows:

30

M: A or C  
R: A or G  
W: A or T  
S: C or G  
Y: C or T  
K: G or T  
V: A or C or G

35

H: A or C or T  
D: A or G or T  
B: C or G or T  
N: G or A or T or C

5

In one embodiment of each of the above-identified aspects of the invention the nucleic acid may comprise a mRNA molecule or alternatively a DNA and preferably a cDNA molecule.

10 Also provided by the present invention is a nucleic acid molecule capable of hybridising to the nucleic acid molecules illustrated in any of Figures 1 to 9 under high stringency conditions such as antisense molecule and which conditions are generally  
15 known to those of skill in the art.

Stringency of hybridisation as used herein refers to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting temperature ( $T_m$ ) of the hybrids.  $T_m$  can be  
20 approximated by the formula:

$$81.5^{\circ}\text{C} + 16.6 (\text{Log}_{10} [\text{Na}^+] + 0.41 (\% \text{G\&C}) - 600 \text{L/L})$$

wherein L is the length of the hybrids in nucleotides.

25  $T_m$  decreases approximately by 1-1.5°C with every 1% decrease in sequence homology.

The term "stringency" refers to the hybridisation conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or  
30 pyrimidine bases therein pair with their corresponding base by hydrogen bonding. High stringency conditions favour homologous base pairing whereas low stringency conditions disfavour non-homologous base pairing.

"Low stringency" conditions comprise, for  
35 example, a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or,

alternatively, a temperature of about 50°C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

"High stringency" conditions comprise, for  
5 example, a temperature of about 42°C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65°C, or less, and a low salt (SSPE) concentration. For example, high stringency  
10 conditions comprise hybridization in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. et al. Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

15 "SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl,  
20 9mM Na<sub>2</sub>HPO<sub>4</sub> and 1 mM EDTA, pH 7.4.

The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the  
25 nucleotide sequences illustrated in any of Figures 1 to 9.

The DNA molecules according to the invention may, advantageously, be included in a suitable expression vector to express polypeptides encoded therefrom in a  
30 suitable host which are critical for growth and survival of *Candida albicans*.

An expression vector according to the invention includes a vector having a nucleic acid according to the invention operably linked to regulatory sequences,  
35 such as promoter regions, that are capable of effecting expression of said DNA fragments. The term

"operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for expression of a polypeptide according to the invention. Thus, in a further aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed, transfected or infected with an expression vector as described above under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

15 The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of said nucleotide and optionally a regulator of the promoter. The vectors may contain one or more  
20 selectable markers, such as, for example, ampicillin resistance.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome  
25 binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or  
30 homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods  
35 well known in the art.

Polynucleotides according to the invention may be inserted into the vectors described in an antisense

orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

In accordance with the present invention, a  
5 defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in bases which result in a synonymous codon (a different codon specifying the same amino acid residue) due to the  
10 degenerate code. The term "nucleic acid sequence" also includes the complementary sequence to any single stranded sequence given regarding base variations.

The present invention also comprises within its scope proteins or polypeptides expressed by the  
15 nucleic acid molecules according to the invention or a functional equivalent, derivative or bioprecursor thereof.

The present invention also advantageously provides nucleic acid sequences of at least  
20 approximately 10 contiguous nucleotides of a nucleic acid according to the invention and preferably from 10 to approximately 120 nucleotides. In another aspect of the invention, nucleotide acid sequences are provided from 10 to 50 nucleotides. These sequences  
25 may, advantageously be used as probes or primers to initiate replication, or the like. Such nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or  
30 the like for detecting the presence of a nucleic acid according to the invention. These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex formation between the probe  
35 and any nucleic acid in the sample.

According to the present invention, these probes may be anchored to a solid support. Preferably, they

- are present on an array so that multiple probes can simultaneously hybridise to a single biological sample. The probes can be spotted onto the array or synthesized *in situ* on the array. See Lockhart et al.,
- 5 Nature Biotechnology, Vol. 14, December 1996, "Expression monitoring by hybridization to high-density oligonucleotide arrays". A single array can contain more than up to more than a million different probes in discrete locations.
- 10 Advantageously, the nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be between
- 15 approximately 10 to 120 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA from a cell, performing a polymerase chain reaction under conditions which bring about amplification of
- 20 the desired region, isolating the amplified region or fragment and recovering the amplified DNA. Generally, such techniques as defined herein are well known in the art, such as described in Sambrook et al (Molecular Cloning: a Laboratory Manual, 1989).
- 25 The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable labels include radioisotopes such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be
- 30 added to the nucleic acids or oligonucleotides of the invention and may be detected using known techniques *per se*.
- The polypeptide or protein according to the invention includes all possible amino acid variants
- 35 encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes.

Polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are substantially homologous to said polypeptides. In  
5 this context, substantial homology is regarded as a sequence which has at least 70%, preferably 80 or 90% amino acid homology with the polypeptides encoded by the nucleic acid molecules according to the invention.

Nucleic acids and polypeptides which are  
10 particularly preferred are those comprising the sequences of nucleotides provided in SEQ ID NOS: 1-3 and polypeptides provided in SEQ ID NOS: 10-12. However, a particularly preferred nucleic acid comprises the sequences of nucleotides provided as SEQ  
15 ID NOS: 2 and/or 3, and their corresponding amino acid sequences identified as SEQ ID NOS: 11 and 12.

With reference to the nucleic acids of this invention and the protein encoded thereby, amino acid sequence SEQ ID NO: 10 is the translation of SEQ ID  
20 NO:1; SEQ ID NO:1 is the translation of SEQ ID NO:2; SEQ ID NO:12 is the translation of SEQ ID NO:3; SEQ ID NO: 16 is the translation of SEQ ID NO:4; SEQ ID NO:13 is the translation of SEQ ID NO:5; SEQ ID NO:14 is the translation of SEQ ID NO:6 and SEQ ID NO:15 is the  
25 translation of SEQ ID NO:7.

Nucleotide sequences according to the invention are particularly advantageous as selective therapeutic targets for treating *Candida albicans* associated infections. For example, an antisense nucleic acid  
30 capable of binding to the nucleic acid sequence illustrated in any of SEQ ID NOS: 1-9 may be used to selectively inhibit expression of the corresponding polypeptides, leading to impaired growth of the *Candida albicans* with reductions of associated  
35 illnesses or diseases (see Figure 5).

The nucleic acid molecule or the polypeptide according to the invention may be used as a

medicament, or in the preparation of a medicament, for treating diseases or conditions associated with *Candida albicans* infection.

- Advantageously, the nucleic acid molecule or the
5. polypeptide according to the invention may be provided in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

- The present invention is further directed to
- 10 inhibiting expression of nucleic acids according to the invention *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation of antisense DNA or RNA, both of which methods are
- 15 based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion or the mature protein sequence, which encodes for the protein of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 50 base pairs in length.
20. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee et al. Nucl. Acids Res., 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991), thereby
- 25 preventing transcription and the production of the corresponding protein. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the corresponding protein (antisense - Okano, J.,
- 30 Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

- Antibodies to the protein or polypeptide of the present invention may, advantageously, be prepared by
- 35 techniques which are known in the art. For example, polyclonal antibodies may be prepared by inoculating a host animal, such as a mouse, with the polypeptide



according to the invention or an epitope thereof and recovering immune serum. Monoclonal antibodies may be prepared according to known techniques such as described by Kohler R. and Milstein C., Nature (1975)  
5 256, 495-497.

Antibodies according to the invention may also be used in a method of detecting for the presence of a polypeptide according to the invention, which method comprises reacting the antibody with a sample and  
10 identifying any protein bound to said antibody. A kit may also be provided for performing said method which comprises an antibody according to the invention and means for reacting the antibody with said sample.

Proteins which interact with the polypeptide of the invention may be identified by investigating  
15 protein-protein interactions using the two-hybrid vector system first proposed by Chien et al. (1991).

This technique is based on functional reconstitution *in vivo* of a transcription factor which  
20 activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating  
25 domain, expressing in the host cell a first hybrid DNA sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the  
30 host at least one second hybrid DNA sequence, such as a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion;  
35 detecting any binding of the proteins to be investigated with a protein according to the invention by detecting for the presence of any reporter gene

product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding sequence, such as for example the nucleic acids according to the invention. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein. Any interaction between polypeptides encoded by the nucleic acid according to the invention and the protein to be tested leads to transcriptional activation of a reporter molecule in a GAL4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as  $\beta$ -galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes.

Further provided by the present invention is one or more *Candida albicans* cells comprising an induced mutation in the DNA sequence encoding the polypeptide according to the invention.

A further aspect of the invention provides a method of identifying compounds which selectively inhibit or interfere with the expression, the functionality of polypeptides expressed from the nucleotides sequences illustrated in any of SEQ ID NOS: 1-9, or the metabolic pathways in which these polypeptides are involved and which are critical for growth and survival of *Candida albicans*, which method comprises (a) contacting a compound to be tested with

one or more *Candida albicans* cells having a mutation in a nucleic acid molecule according to the invention which mutation results in overexpression or underexpression of said polypeptides in addition to  
5 one or more wild type *Candida* cells, (b) monitoring the growth and/or activity of said mutated cell compared to said wild type wherein differential growth or activity of said one or more mutated *Candida* cells provides an indication of selective action of said  
10 compound on said polypeptide or another polypeptide in the same or a parallel pathway.

Compounds identifiable or identified using the method according to the invention, may advantageously be used as a medicament, or in the preparation of a  
15 medicament to treat diseases or conditions associated with *Candida albicans* infection. These compounds may also advantageously be included in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

20 A further aspect of the invention provides a method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival, which method comprises (a) preparing a cDNA or genomic library from  
25 said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library, (b)  
30 selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant. Preferably, the cell or organism may be any yeast or filamentous fungus,  
35 such as, for example, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

A further aspect of the invention provides a

pharmaceutical composition comprising any of a compound, an antisense molecule or an antibody according to the invention together with a pharmaceutically acceptable carrier, diluent or  
5 excipient therefor.

The antisense molecules or indeed the compounds identified as agonists or antagonists of the nucleic acids or polypeptides according to the invention may be used in the form of a pharmaceutical composition,  
10 which may be prepared according to procedures well known in the art. Preferred compositions include a pharmaceutically acceptable vehicle or diluent or excipient, such as for example, a physiological saline solution. Other pharmaceutically acceptable carriers  
15 including other non-toxic salts, sterile water or the like may also be used. A suitable buffer may also be present allowing the compositions to be lyophilized and stored in sterile conditions prior to reconstitution by the addition of sterile water for  
20 subsequent administration. Incorporation of the polypeptides of the invention into a solid or semi-solid biologically compatible matrix may be carried out which can be implanted into tissues requiring treatment.

25 The carrier can also contain other pharmaceutically acceptable excipients for modifying other conditions such as pH, osmolarity, viscosity, sterility, lipophilicity, solubility or the like. Pharmaceutically acceptable excipients which permit  
30 sustained or delayed release following administration may also be included.

The polypeptides, the nucleic acid molecules or compounds according to the invention may be administered orally. In this embodiment they may be  
35 encapsulated and combined with suitable carriers in solid dosage forms which would be well known to those skilled in the art.

As would be well known to those of skill in the art, the specific dosage regime may be calculated according to the body surface area of the patient or the volume of body space to be occupied, dependent  
5 upon the particular route of administration to be used. The amount of the composition actually administered will, however, be determined by a medical practitioner, based on the circumstances pertaining to the disorder to be treated, such as the severity of  
10 the symptoms, the composition to be administered, the age, weight, and response of the individual patient and the chosen route of administration.

#### Example 1

#### 15 **Identification of novel drug targets in *C. albicans* by anti-sense and disruptive integration**

The principle of the approach is based on the fact that when a particular *C. albicans* mRNA is inhibited by producing the complementary anti-sense  
20 RNA, the corresponding protein will decrease. If this protein is critical for growth or survival, the cell producing the anti-sense RNA will grow more slowly or will die.

Since anti-sense inhibition occurs at mRNA level,  
25 the gene copy number is irrelevant, thus allowing applications of the strategy even in diploid organisms.

Anti-sense RNA is endogenously produced from an integrative or episomal plasmid with an inducible  
30 promoter; induction of the promoter leads to the production of an RNA encoded by the insert of the plasmid. This insert will differ from one plasmid to another in the library. The inserts will be derived from genomic DNA fragments or from cDNA to cover-to  
35 the extent possible- the entire genome.

The vector is a proprietary vector allowing

integration by homologous recombination at either the homologous insert or promoter sequence in the *Candida* genome. After introducing plasmids from cDNA or genomic libraries into *C. albicans*, transformants are  
5 screened for impaired growth after promoter (& thus anti-sense) induction in the presence of lithium acetate. Lithium acetate prolongs the G1 phase and thus allows anti-sense to act during a prolonged period of time during the cell cycle. Transformants  
10 which show impaired growth in both induced and non-induced media, thus showing a growth defect due to integrative disruption, are selected as well.

Transformants showing impaired growth are supposed to contain plasmids which produce anti-sense  
15 RNA to mRNAs critical for growth or survival. Growth is monitored by measuring growth-curves over a period of time in a device (Bioscreen Analyzer, Labsystems) which allows simultaneous measurement of growth-curves of 200 transformants.

20 Subsequently plasmids can be recovered from the transformants and the sequence of their inserts determined, thus revealing which mRNA they inhibit. In order to be able to recover the genomic or cDNA insert which has integrated into the *Candida* genome, genomic  
25 DNA is isolated, cut with an enzyme which cuts only once into the library vector (and estimated approx. every 4096 bp in the genome) and religated. PCR with primers flanking the insert will yield (partial) genomic or cDNA inserts as PCR fragments which can  
30 directly be sequenced. This PCR analysis (on ligation reaction) will also show us how many integrations occurred. Alternatively the ligation reaction is transformed to *E. coli* and PCR analysis is performed on colonies or on plasmid DNA derived thereof.

35 This method is employed for a genome-wide search for novel *C. albicans* genes which are important for growth or survival.

## Materials & Methods

### Construction of pGAL1PNiST-1 (SEQ ID NO:21)

The backbone of the pGAL1PNiST-1 vector  
5 (integrative anti-sense *SfiI*-*NotI* vector) is  
pGEM11Zf(+) (Promega Inc.). First, the CaMAL2  
*EcoRI*/*SalI* promoter fragment from pDBV50 (D.H. Brown  
et al. 1996) was ligated into *EcoRI*/*SalI*-opened  
pGEM11Zf(+) resulting in the intermediate construct  
10 pGEMMAL2P-1. Into the latter (*MscI*/*CIP*) the CaURA3  
selection marker was cloned as a *Eco47III*/*XmnI*  
fragment derived from pRM2. The resulting pGEMMAL2P-2  
vector was *NotI*/*HindIII* opened in order to accept the  
*NotI*-stuffer-*SfiI* cassette from pPCK1NiSCYCT-1  
15 (*EagI*/*HindIII* fragment): pMAL2PNiST-1. Finally, the  
plasmid pGAL1PNiST-1 was constructed by exchanging the  
*SalI*/*Ecl136II* MAL2 promoter in pMAL2PNiST-1 by the  
*XhoI*/*SmaI* GAL1 promoter fragment derived from  
pRM2GAL1P.

20

### Construction of pGAL1PSiST-1 (SEQ ID ON:22)

The vector pGAL1PSiST-1 was created for cloning  
the small genomic DNA fragments (flanked by *SfiI*  
sites) behind the GAL1 promoter. The only difference  
25 with pGAL1PNiST-1 is that the hIFN $\beta$  (stuffer fragment)  
insert fragment in pGAL1PSiST-1 is flanked by two *SfiI*  
sites in stead of a *SfiI* and a *NotI* site as in  
pGAL1PNiST-1. To construct pGAL1PSiST-1 the *EcoRI*-  
*HindIII* fragment, containing hIFN $\beta$  flanked by a *SfiI*  
30 and a *NotI* site, of pMAL2pHiET-3 (unpublished) was  
exchanged by the *EcoRI*-*HindIII* fragment, containing  
hIFN $\beta$  flanked by two *SfiI* sites, from YCp50S-S (an *E.*  
*coli* / *S. cerevisiae* shuttle vector derived from the  
plasmid YCp50, which is deposited in the ATCC  
35 collection (number 37419; Thrash et al., 1985); an

*EcoRI-HindIII* fragment, containing the gene *hIFN $\beta$* , which is flanked by two *SfiI* sites, was inserted in YCp50, creating YCp50S-S), resulting into plasmid pMAL2PSiST-1. The *MAL2* promoter from pMAL2PSiST-1 (by  
5 a *NaeI-balI* digest) was further replaced by the *GAL1* promoter from pGAL1PNiST-1 (via a *XhoI-FSPI* digest), creating the vector pGAL1PSiST-1.

### ***Candida albicans* genomic library**

#### 10       \* Preparation of the genomic DNA fragments

A *Candida albicans* genomic DNA library with small DNA fragments (400 to 1,000 bp) was prepared. Genomic DNA of *Candida albicans* B2630 was isolated following a modified protocol of Blin and Stafford (1976). The  
15 quality of the isolated genomic DNA was checked by gel electrophoresis. Undigested DNA was located on the gel above the marker band of 26,282 bp. A little smear, caused by fragmentation of the DNA, was present. To obtain enrichment for genomic DNA fragments of the  
20 desired size, the genomic DNA was partially digested. Several restriction enzymes (*AluI*, *HaeIII* and *RsaI*; all creating blunt ends) were tried out. The appropriate digest conditions have been determined by titration of the enzyme. Enrichment of small DNA  
25 fragments was obtained with 70 units of *AluI* on 10  $\mu$ g of genomic DNA for 20 min. T4 DNA polymerase (Boehringer) and dNTPs (Boehringer) were added to polish the DNA ends. After extraction with phenol-chloroform the digest was size-fractionated on an  
30 agarose gel. The genomic DNA fragments with a length of 500 to 1,250 bp were eluted from the gel by centrifugal filtration (Zhu et al., 1985). *SfiI* adaptors (5' GTTGGCCTTTT) or (5' AGGCCAAC) were attached to the DNA ends (blunt) to facilitate cloning  
35 of the fragments into the vector. Therefore, a 8-mer and 11-mer oligonucleotide (comprising the *SfiI* site)



were kinased and annealed. After ligation of these adaptors to the DNA fragments a second size-fractionation was performed on an agarose gel. The DNA fragments of 400 to 1150 bp were eluted from the gel by centrifugal filtration.

\* *Preparation of the pGAL1PSiST-1 vector fragment*

The small genomic DNA fragments were cloned after the GAL1 promoter in the vector pGAL1PSiST-1. Qiagen-purified pGAL1PSiST-1 plasmid DNA was digested with *SfiI* and the largest vector fragment eluted from the gel by centrifugal filtration (Zhu *et al.*, 1985). Ligation with a control DNA fragment, flanked by *SfiI* sites, was performed as a control. The ligation mix was electroporated to MC1061 *E. coli* cells. Plasmid DNA of 24 clones was analyzed. In all cases the control fragment was inserted in the pGAL1PSiST-1 vector fragment.

\* *Upscaling*

All genomic DNA fragments (450 ng) were ligated into the pGAL1PSiST-1 vector (20-ng). After electroporation at 2500V, 40 $\mu$ F circa 400,000 clones were obtained. These clones were pooled into three groups and stored as glycerol slants. Also Qiagen-purified DNA was prepared from these clones. A clone analysis showed an average insert length of 600 bp and a percentage of 91 for clones with an insert. The size of the library corresponds to 5 times the diploid genome. The genomic DNA inserts are sense or anti-sense orientated in the vector.

***Candida albicans* cDNA library**

Total RNA was extracted from *Candida albicans* B2630 grown on respectively minimal (SD) and rich (YPD) medium as described by Chirgwin *et al.* in Sambrook *et al* 1996. mRNA was prepared from total RNA using the Invitrogen Fast Track procedure.

First strand cDNA is synthesised with the

Superscript Reverse Transcriptase (BRL) and with an oligo dT-NotI Primer adapter. After second strand synthesis, cDNA is polished with Klenow enzyme and purified over a Sephacryl S-400 spun column.

- 5 Phosphorylated *SfiI* adapters are then ligated to the cDNA, followed by digestion with the *NotI* restriction enzyme. The *SfiI*/*NotI* cDNA is then purified and sized on a Biogel column A150M.

- First fraction contains approximately 38,720  
10 clones by transformation, the second fraction only 1540 clones. Clone analysis:

Fr. I: 22/24 inserts, 16 ; 1000 bp, 4 ; 2000 bp, average size: 1500 bp.

Fr. II: 9/12 inserts, 3 ; 1000 bp, average size: 960

- 15 bp cDNA was ligated in a *NotI*/*SfiI* opened pGAL1PNiST-1 vector (anti-sense)

#### ***Candida* transformation**

- The host strain used for transformation is a *C. albicans* *ura3* mutant, CAI-4, which contains a deletion  
20 in orotidine-5-phosphate decarboxylase and was obtained from William Fonzi, Georgetown University (Fonzi and Irwin). CAI-4 was transformed with the above described cDNA library or genomic library using  
25 the *Pichia* spheroplast module (Invitrogen). Resulting transformants were plated on minimal medium supplemented with glucose (SD, 0.67% or 1.34% Yeast Nitrogen base w/o amino acids + 2% glucose) plates and incubated for 2-3 days at 30°C.

30

#### **Screening for mutants**

- Starter cultures were set up by inoculating each colony in 1 ml SD medium and incubating overnight at 30°C and 300 rpm. Cell densities were determined using  
35 a Coulter counter (Coulter Z1; Coulter electronics limited). 250.000 cells/ml were inoculated in 1 ml SD

medium and cultures were incubated for 24 hours at 30°C and 300 rpm. Cultures were washed in minimal medium without glucose (S) and the pellet resuspended in 650 µl S medium. 8 µl of this culture is used for  
5 inoculating 400 µl cultures in a Honeywell-100 plate (Bioscreen analyzer; Labsystems). Each transformant was grown during three days in S medium containing LiAc; pH 6.0, with 2% glucose/2% maltose or 2% galactose/2% maltose respectively while shaking every  
10 3 minutes for 20 seconds. Optical densities were measured every hour during three consecutive days and growth curves were generated (Bioscreen analyzer; Labsystems).

Growth curves of transformants grown in  
15 respectively anti-sense non-inducing (glucose/maltose) and inducing (galactose/maltose) medium are compared and those transformants showing impaired growth upon anti-sense induction are selected for further analysis. Transformants showing impaired growth by  
20 virtue of integration into a critical gene are also selected.

#### Isolation of genomic or cDNA inserts

Putatively interesting transformants are grown in  
25 1.5 ml SD overnight and genomic DNA is isolated using the Nucleon MI Yeast kit (Clontech). Concentration of genomic DNA is estimated by analyzing a sample on an agarose gel.

20 ng of genomic DNA is digested for three hours  
30 with an enzyme that cuts uniquely in the library vector (SacI for the genomic library; PstI for the cDNA library) and treated with RNase. Samples are phenol/chloroform extracted and precipitated using NaOAc/ethanol.

35 The resulting pellet is resuspended in 500 µl ligation mixture (1 x ligation buffer and 4 units of

T4 DNA ligase; both from Boehringer) and incubated overnight at 16°C.

After denaturation (20 min 65°C), purification (phenol/chloroform extraction) and precipitation (NaOAc/ethanol) the pellet is resuspended in 10 µl MilliQ (Millipore) water.

#### PCR analysis

Inverse PCR is performed on 1 µl of the precipitated ligation reaction using library vector specific primers (oligo23 5' TGC-AGC-TCG-ACC-TCG-ACT-G 3' SEQ ID NO: 17 and oligo25 5' GCG-TGA-ATG-TAA-GCG-TGA-C 3' SEQ ID NO: 18 for the genomic library; 3pGALNistPCR primer: 5'TGAGCAGCTCGCCGTCGCGC 3' SEQ ID NO: 19 and 5pGALNistPCR primer: 5'GAGTTATACCCTGCAGCTCGAC 3' SEQ ID NO: 20 for the cDNA library; both from Eurogentec) for 30 cycles each consisting of (a) 1 min at 95 °C, (b) 1 min at 57 °C, and (c) 3 min at 72 °C. In the reaction mixture 2.5 units of Taq polymerase (Boehringer) with TaqStart antibody (Clontech) (1:1) were used, and the final concentrations were 0.2 µM of each primer, 3 mM MgCl<sub>2</sub> (Perkin Elmer Cetus) and 200 µM dNTPs (Perkin Elmer Cetus). PCR was performed in a Robocycler (Stratagene).

#### Sequence determination

Resulting PCR products were purified using PCR purification kit (Qiagen) and were quantified by comparison of band intensity on EtBr stained agarose gel with the intensity of DNA marker bands. The amount of PCR product (expressed in ng) used in the sequencing reaction is calculated as the length of the PCR product in basepairs divided by 10. Sequencing reactions were performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit

according to the instructions of the manufacturer (PE Applied Biosystems, Foster City, CA) except for the following modifications.

- The total reaction volume was reduced to 15  $\mu$ l.
- 5 Reaction volume of individual reagents were changed accordingly. 6.0  $\mu$ l Terminator Ready Reaction Mix was replaced by a mixture of 3.0  $\mu$ l Terminator Ready Reaction Mix + 3.0  $\mu$ l Half Term (GENPAK Limited, Brighton, UK). After cycle sequencing, reaction
- 10 mixtures were purified over Sephadex G50 columns prepared on Multiscreen HV opaque microtiter plates (Millipore, Molsheim, Fr) and were dried in a speedVac. Reaction products were resuspended in 3  $\mu$ l loading buffer. Following denaturation for 2 min at
- 15 95°C, 1  $\mu$ l of sample was applied on a 5% Long Ranger Gel (36 cm well-to-read) prepared from Singel Packs according to the supplier's instructions (FMC BioProducts, Rockland, ME). Samples were run for 7 hours 2X run on a ABI 377XL DNA sequencer. Data
- 20 collection version 2.0 and Sequence analysis version 3.0 (for basecalling) software packages are from PE Applied Biosystems. Resulting sequence text files were copied onto a server for further analysis.

## 25 **Sequence analysis**

- Nucleotide sequences were imported in the VectorNTI software package (InforMax Inc, North Bethesda, MD, USA), and the vector and insert regions of the sequences were identified. Sequence similarity
- 30 searches against public and commercial sequence databases were performed with the BLAST software package (Altschul et al., 1990) version 1.4. Both the original nucleotide sequence and the six-frame conceptual translations of the insert region were used
- 35 as query sequences. The used public databases were the EMBL nucleotide sequence database (Stoesser et al.,

1998), the SWISS-PROT protein sequence database and its supplement TrEMBL (Bairoch and Apweiler, 1998), and the ALCES *Candida albicans* sequence database (Stanford University, University of Minnesota). The commercial sequence databases used were the LifeSeq<sup>®</sup> human and PathoSeq<sup>®</sup> microbial genomic databases (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA), and the GENESEQ patent sequence database (Derwent, London, UK). Three major results were obtained on the basis of the sequence similarity searches: function, novelty, and specificity. A putative function was deduced on the basis of the similarity with sequences with a known function, the novelty was based on the absence or presence of the sequences in public databases, and the specificity was based on the similarity with vertebrate homologues.

#### Methods

Blastx of the nucleic acid sequences against the appropriate protein databases: Swiss-Prot for clones of which the complete sequence is present in the public domain, and paorfp (PathoSeq<sup>™</sup>) for clones of which the complete sequences is not present in the public domain.

The protein to which the translated nucleic acid sequence corresponds to is used as a starting point. The differences between this protein and our translated nucleic acid sequences are marked with a double line and annotated above the protein sequence.

The following symbols are used:

A one-letter amino acid code or the ambiguity code X is used if our translated nucleic acid sequence has another amino acid on a certain position,

The stop codon sign \* is used if our translated nucleic acid sequence has a stop codon on a certain position,

The letters fs (frame shift) are used if a frame

shift occurs in our translated nucleic acid sequence,  
and another reading frame is used,

The words ambiguity or ambiguities are used if a  
part of our translated nucleic acid sequence is  
5 present in the proteins, but not visible in the  
alignments of the blast results,

The phrase "missing sequence" is used if the  
translated nucleic acid sequence does not comprise  
that part of the protein.

10 Blastx: compares the six-frame conceptual  
translation products of a nucleotide query sequence  
(both strands) against a protein sequence database.

### Antisense Experiments

15

Clones 383c\_cp, 392c\_cp, 417c\_cpG2L, 325c\_af,  
322c\_cp, 26g3, and 409c\_cp were transformed with  
plasmid pGAL1PSiST-1 containing the galactose  
inducible promotor/expression cassette. The plasmid  
20 was modified to include antisense molecules capable of  
binding to RNA expressed from DNA corresponding to SEQ  
ID NOs: 1-2 and SEQ ID NOs 4-8 natively present the  
*Candida albicans* cell. Following transformation the  
cells were grown in the presence of glucose or  
25 galactose to determine the effect of the induced  
antisense molecule on protein expression and on  
*Candida albicans* cell growth. An exemplary growth  
curve is provided in Figure 5. All cells transformed  
with the antisense molecules demonstrated significant  
30 growth reduction.

### Gene Knock-outs

To verify that the growth effect was due to the  
interference with the identified gene and to support  
35 the specificity of the antisense effect, single and  
double allele knock-outs were made in the identified  
genes using the URA-blaster method (Fonzi and Irwin

1993). Figure 4 provides an example of a growth curve for a double allele knock-out as compared to the wild-type strain CAI4. Growth curves are shown as the measurement of optical density (Y-axis) over time in 5 hours (X-axis). The optical density is a measure for the number of cells present and this a measure for growth. A reduction in growth was observed for this double knock-out as compared to the wild-type strain.

10 **Screening for compounds modulating expression of**  
**polypeptides critical for growth and survival of *C.***  
***albicans***

The method proposed is based on observations (Sandbaken et al., 1990; Hinnebusch and Liebman 1991; 15 Ribogene PCT WO 95/11969, 1995) suggesting that underexpression or overexpression of any component of a process (e.g. translation) could lead to altered sensitivity to an inhibitor of a relevant step in that process. Such an inhibitor should be more potent 20 against a cell limited by a deficiency in the macromolecule catalyzing that step and/or less potent against a cell containing an excess of that macromolecule, as compared to the wild type (WT) cell.

Mutant yeast strains, for example, have shown 25 that some steps of translation are sensitive to the stoichiometry of macromolecules involved. (Sandbaken et al. 1996). Such strains are more sensitive to compounds, which specifically perturb translation (by acting on a component that participates in 30 translation) but are equally sensitive to compounds with other mechanisms of action.

This method thus not only provides a means to identify whether a test compound perturbs a certain process but also an indication of the site at which it 35 exerts its effect. The component which is present in altered form or amount in a cell whose growth is



affected by a test compound is potentially the site of action of the test compound.

The assay involves measurement of growth of an isogenic strain which has been modified only in a certain specific allele, relative to a wild type (WT) *C. albicans* strain, in the presence of R-compounds. Strains can be ones in which the expression of a specific essential protein is impaired upon induction of anti-sense or strains which carry disruptions in an essential gene. An *in silico* approach to finding novel essential genes in *C. albicans* will be performed. A number of essential genes identified in this way will be disrupted (in one allele) and the resulting strains can be used for comparative growth screening.

#### Assay for High Throughput screening for drugs

35  $\mu$ l minimal medium (S medium + 2% galactose + 2% maltose) is transferred in a transparent flat-bottomed 96 well plate using an automated pipetting system (Multidrop, Labsystems). A 96-channel pipettor (Hydra, Robbins Scientific) transfers 2.5  $\mu$ l of R-compound at  $10^{-3}$  M in DMSO from a stock plate into the assay plate.

25 The selected *C. albicans* strains (mutant and parent (CAI-4) strain) are stored as glycerol stocks (15%) at  $-70^{\circ}\text{C}$ . The strains are streaked out on selective plates (SD medium) and incubated for two days at  $30^{\circ}\text{C}$ . For the parent strain, CAI-4, the medium is always supplemented with 20  $\mu\text{g/ml}$  uridine. A single colony is scooped up and resuspended in 1 ml minimal medium (S medium + 2% galactose + 2% maltose). Cells are incubated at  $30^{\circ}\text{C}$  for 8 hours while shaking at 250 rpm. A 10 ml culture is inoculated at 250,000 cells/ml. Cultures are incubated at  $30^{\circ}\text{C}$  for 24 hours while shaking at 250 rpm. Cells are counted in Coulter counter and the final culture (S medium + 2%

- Optical densities are measured after 48 hours.

- 10        Test growth is expressed as a percentage of  
positive control growth for both mutant (x) and wild  
type (y) strains. The ratio (x/y) of these derived  
variables is calculated.

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**Table 1**

Seq ID No.	Clone	Function
1	382c_cp	-
2	392c_cp	TUF1

3	-	RAD53
4	417c_cpG2L	-
5	325c_af	-
6	322c_cp <sup>1</sup>	-
7	26g3	-
8	409c_cp	-
9	382c_cpG1L2	-
10	382c_cp (prt)	-
11	392c_cp (prt)	TUF1
12		RAD53
13	325c_af (prt) <sup>2</sup>	-
14	322c_cp (prt) <sup>2</sup>	-
15	26g3 (prt)	-
16	417c_cp 92L (prt)	-

1. 322c-cp is a member of the UPF0057 protein family. It contains potential transmembrane regions (6-23aa; 30-53aa) and could be low temperature or salt-stress inducible.
2. 325c-af shows similarity to IMP4 yeast and related proteins and it might be involved in rRNA processing in *Candida albicans* in a similar way to IMP4.

What is claimed is:

1. A nucleic acid molecule encoding a polypeptide  
which is critical for survival and growth of the yeast  
5 *Candida albicans* and which nucleic acid molecule is  
selected from the group consisting of SEQ ID NO: 1 to  
SEQ ID NO: 9.
2. A nucleic acid molecule encoding a polypeptide  
10 which is critical for survival and growth of the yeast  
*Candida albicans* and which nucleic acid molecule is  
selected from the group consisting of SEQ ID NO: 1 to  
SEQ ID NO: 3.
- 15 3. A nucleic acid molecule encoding a polypeptide  
which is critical for survival and growth of the yeast  
*Candida albicans* and which nucleic acid molecule is  
selected from SEQ. ID NO: 1 or SEQ. ID. NO: 2 and  
fragments or derivatives of said nucleic acid  
20 molecules.
4. The nucleic acid molecule of Claim 1 which is mRNA.
5. The nucleic acid molecule of Claim 1 which is DNA.
- 25 6. The nucleic acid molecule according to claim 5  
which is cDNA.
7. A first nucleic acid molecule capable of  
30 hybridising to a second nucleic acid molecule encoding  
a polypeptide which is critical for survival and  
growth of the yeast *Candida albicans* and which second  
nucleic acid molecule is selected from the group  
consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein  
35 hybridisation occurs under high stringency conditions.
8. An antisense molecule comprising a nucleic acid

molecule capable of hybridising to a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* wherein the nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

9. Cells containing a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein said cells are bacterial or eukaryotic.

10. A polypeptide encoded by a nucleic acid molecule which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

11. A polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID NO: 16.

12. A recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein the nucleic acid molecule is DNA.

13. A recombinant DNA construct comprising a first nucleic acid molecule wherein the first nucleic acid molecule or a molecule complementary to the first nucleic acid molecule is capable of hybridizing a second nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which second nucleic acid

molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9 wherein the first nucleic acid molecule is inserted in the antisense orientation.

5

14. A recombinant DNA construct according to claim 12 wherein said recombinant DNA construct is an expression vector.

10 15. The construct according to claim 14 which comprises an inducible promoter.

16. The construct according to claim 14 which comprises a sequence encoding a reporter molecule.

15

17. Cells containing a recombinant DNA construct comprising a nucleic acid molecule encoding a polypeptide which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, wherein said cells are bacterial or eukaryotic.

20

18. The nucleic acid molecule according to claim 1 in a pharmaceutically acceptable carrier, diluent, excipient or buffer.

25

19. A method for treating a *Candida albicans*-associated disease comprising the step of:

30 administering a composition of matter comprising an antisense nucleic acid molecule capable of binding to a *Candida albicans*-originating nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9.

35

20. A pharmaceutical composition comprising a polypeptide according to claim 10 together with a

pharmaceutically acceptable carrier diluent or excipient therefor.

21. A *Candida albicans* cell comprising an induced  
5 mutation in the DNA sequence encoding the polypeptide according to claim 10.

22. A method of identifying compounds which  
selectively modulate expression or functionality of  
10 polypeptides or metabolic pathways in which these polypeptides are involved and which are crucial for growth and survival of *Candida albicans*, which method comprises:

(a) contacting a compound to be tested with one  
15 or more *Candida albicans* cells having a mutation in a nucleic acid molecule according to claim 1 which mutation results in overexpression or underexpression of said polypeptides in addition to contacting one  
20 or more wild type *Candida albicans* cells with said compound; and

(b) monitoring the growth and/or activity of  
said mutated cell compared to said wild  
25 type; wherein differential growth or activity of said one or more mutated *Candida* cells is indicative of selective action of said compound on a polypeptide or another polypeptide in the same or a parallel pathway.

30

23. A compound identifiable according to the method of claim 22.

24. A pharmaceutical composition comprising a  
35 compound according to claim 23 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

25. A method of identifying DNA sequences from a cell or organism which DNA encodes polypeptides which are critical for growth or survival of said cell or

5 organism, which method comprises:

- 10 (a) preparing a cDNA or genomic library from said cell or organism in a suitable expression vector which vector is such that it can either integrate into the genome in said cell or that it permits transcription of antisense RNA from the nucleotide sequences in said cDNA or genomic library; and
- 15 (b) selecting transformants exhibiting impaired growth and determining the nucleotide sequence of the cDNA or genomic sequence from the library included in the vector from said transformant.

-----20 26. The method according to claim 25 wherein said cell or organism is a yeast or filamentous fungus.

27. The method according to claim 25 wherein said cell or organism is selected from the group consisting  
25 of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* or *Candida albicans*.

28. An antibody capable of binding to a polypeptide encoded by a nucleic acid molecule which is critical  
30 for survival and growth of the yeast *Candida albicans* and which nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9.

29. An oligonucleotide comprising a fragment of from  
35 10 to 120 contiguous nucleotides of a nucleic acid molecule which is critical for survival and growth of the yeast *Candida albicans* and which nucleic acid

molecule is selected from the group consisting of SEQ  
ID NO: 1 to SEQ ID NO: 9.

30. The oligonucleotide according to claim 29  
5 comprising a fragment of from 10 to 50 contiguous  
nucleotides.



ABSTRACT**DRUG TARGETS IN *CANDIDA ALBICANS***

5

The present invention is concerned with the identification of genes or functional fragments thereof from *Candida albicans* which are critical for growth and cell division and which genes may be used  
10 as selective drug targets to treat *Candida albicans* associated infections. Novel nucleic acid sequences from *Candida albicans* are also provided and which encode the polypeptides which are critical for growth of *Candida albicans*. Methods for the identification of  
15 anti-fungal compounds which inhibit fungal or yeast growth are also contemplated.

FIGURE 1

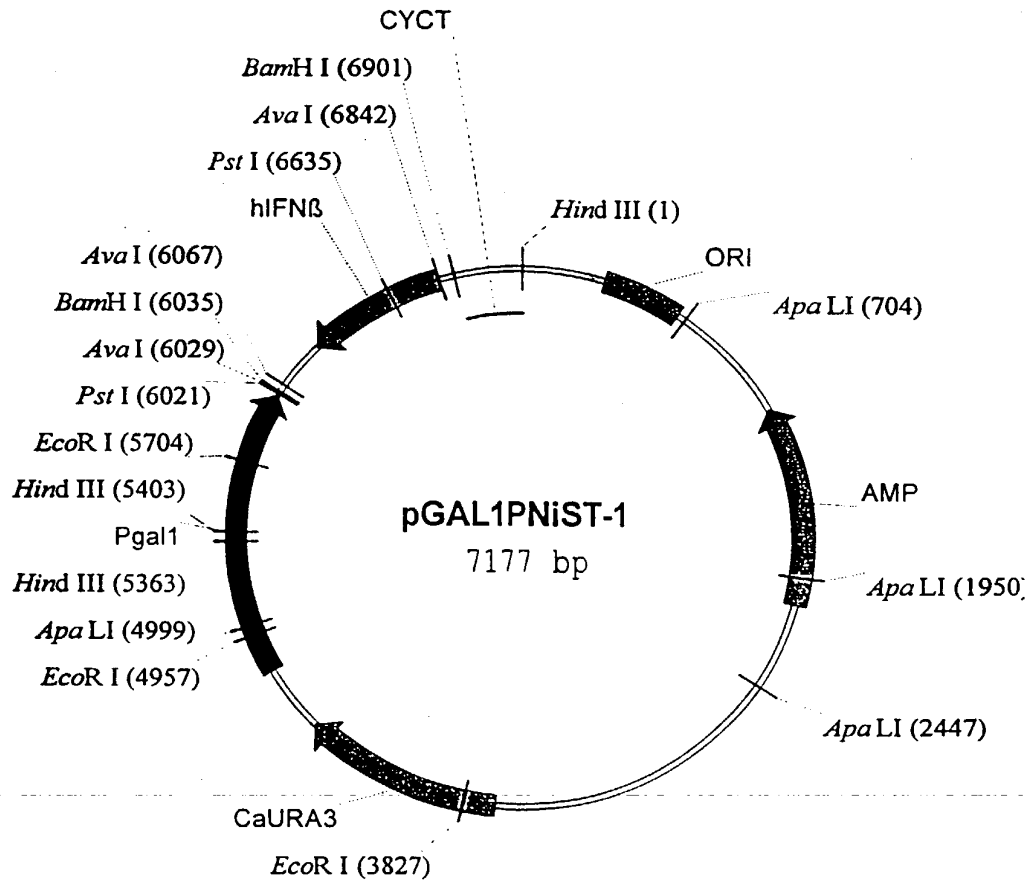


FIGURE 2

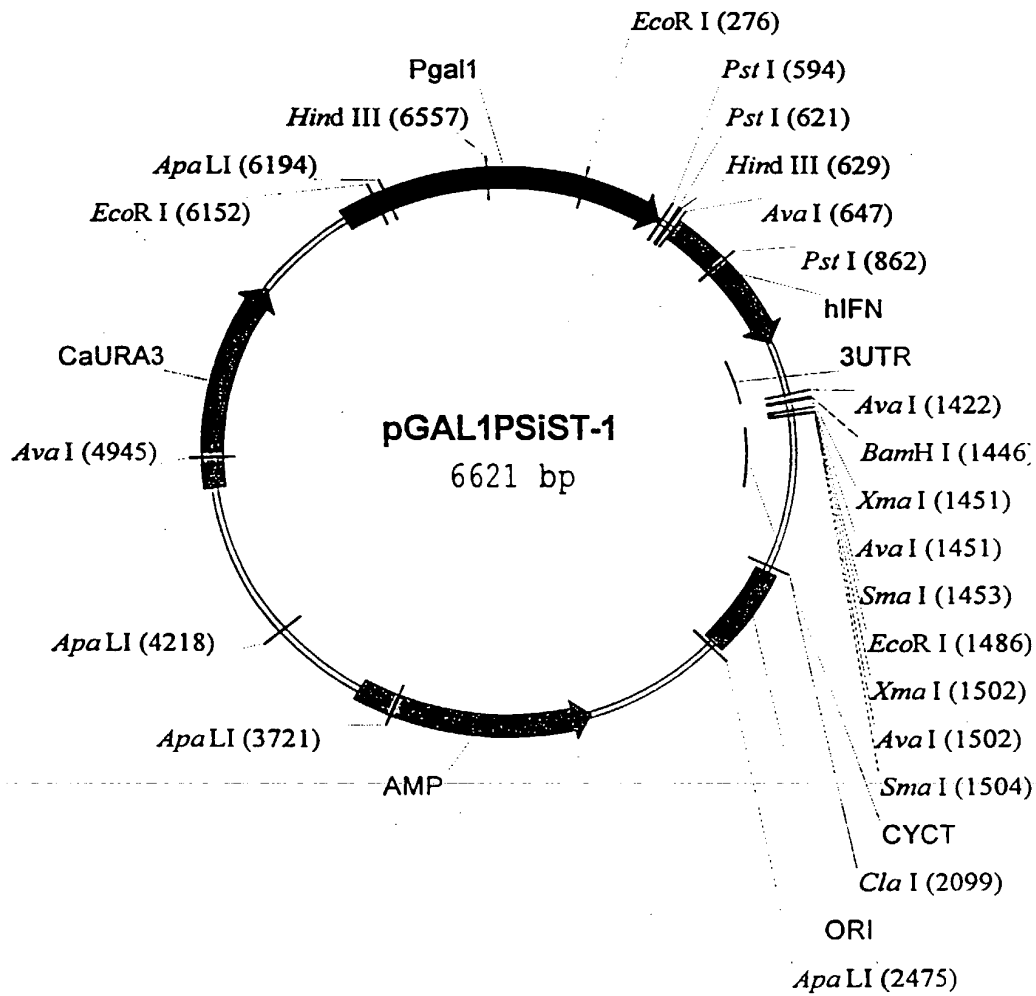


FIGURE 3

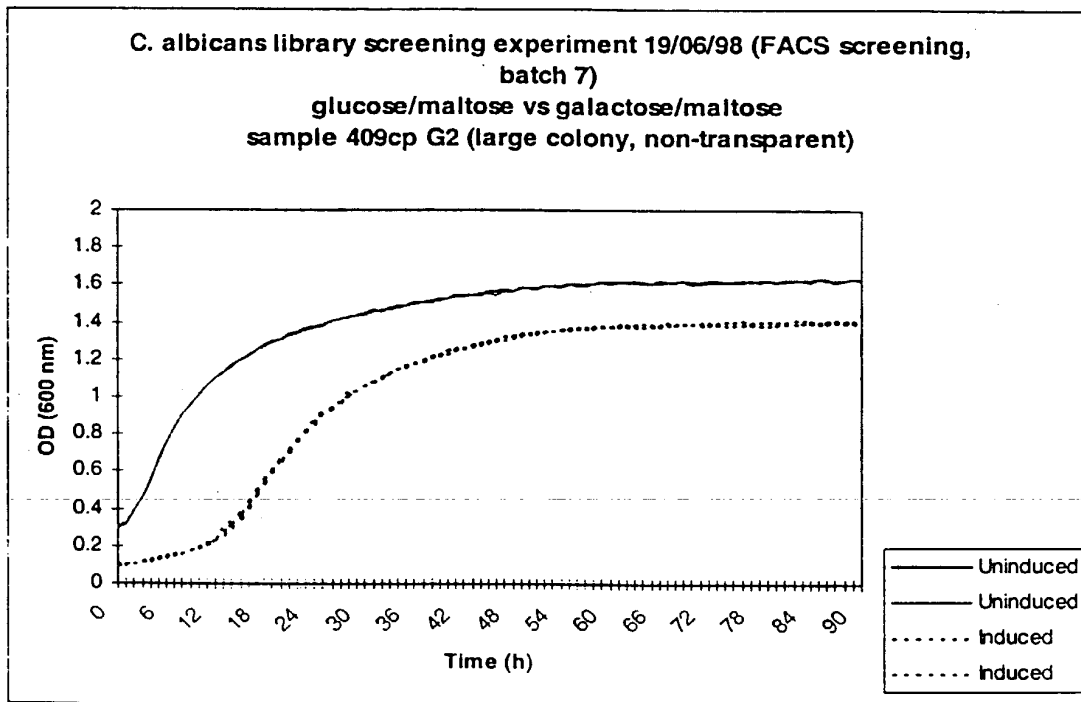
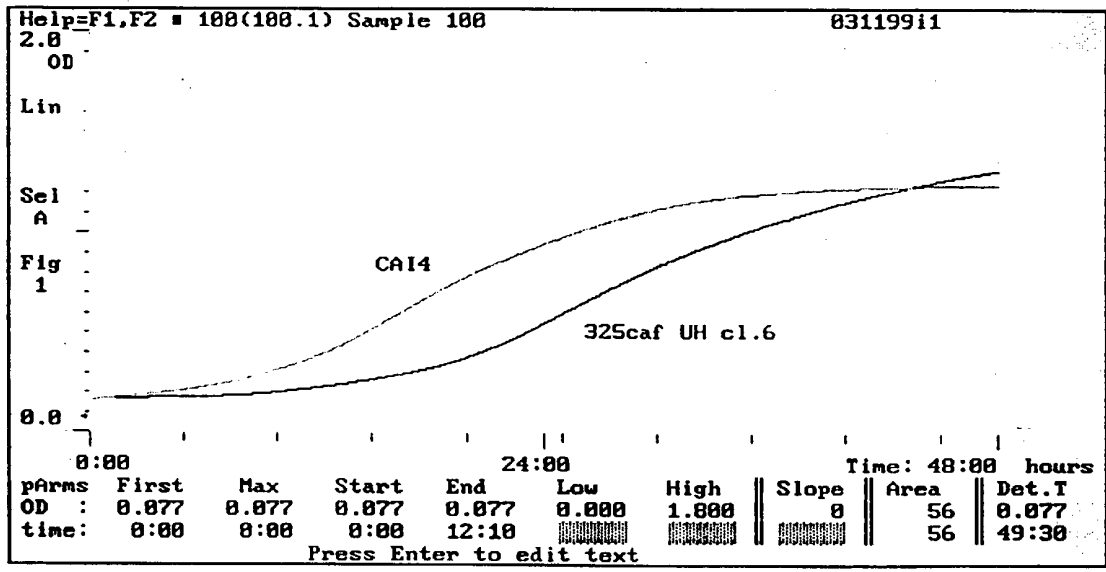


FIGURE 4



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	<b>First Named Inventor</b>	Contreras, Roland Henri
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	<b>Application Number</b>	/
	<b>Filing Date</b>	
	<b>Group Art Unit</b>	
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**As a below named inventor, I hereby declare that:**

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**DRUG TARGETS IN CANDIDA ALBICANS**

the specification of which (Title of the Invention)

☐ is attached hereto  
OR  
☐ was filed on (MM/DD/YYYY) **12/06/1999** as United States Application Number or PCT International Application Number **PCT/EP99/09833** and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

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98204122.0	EP	12/04/1998	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

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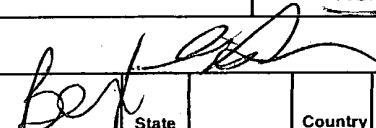
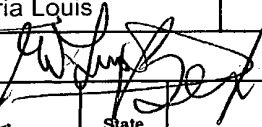
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Inventor's Signature				Date		03/20/01	
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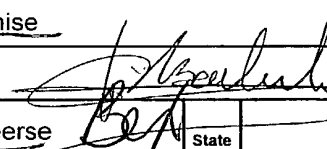
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**DECLARATION**
**ADDITIONAL INVENTOR(S)**  
**Supplemental Sheet**  
 Page 1 of 2

<b>Name of Additional Joint Inventor, if any:</b>		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Bart Jozef Maria				Nelissen			
Inventor's Signature						Date	
Residence: City	Beerse	State		Country	Belgium	Citizenship	BE
Post Office Address	c/o Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium						
Post Office Address							
City	Meerhout	State		ZIP	2450	Country	Belgium
<b>Name of Additional Joint Inventor, if any:</b>		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
3-80 Marianne Denise				De Backer			
Inventor's Signature						Date	3/15/2001
Residence: City	Beerse	State		Country	Belgium	Citizenship	BE
Post Office Address	c/o Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium						
Post Office Address							
City	San Diego	State	CA	ZIP	92122	Country	USA
<b>Name of Additional Joint Inventor, if any:</b>		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])				Family Name or Surname			
Walter Herman Maria Louis				Luyten			
Inventor's Signature						Date	
Residence: City	Beerse	State		Country	Belgium	Citizenship	BE
Post Office Address	c/o Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium						
Post Office Address							
City	Turnhout	State		ZIP	2300	Country	Belgium

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**DECLARATION**
**ADDITIONAL INVENTOR(S)**  
**Supplemental Sheet**  
 Page 2 of 2

<b>Name of Additional Joint Inventor, if any:</b>		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle (if any))		Family Name or Surname					
Jasmine Elza		Viaene					
Inventor's Signature						Date	03/22/01
Residence: City	Gent	State		Country	Belgium	Citizenship	BE
Post Office Address	c/o University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium						
Post Office Address							
City	Varsenare	State		ZIP	8490	Country	Belgium
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Given Name (first and middle (if any))		Family Name or Surname					
Marc George		Logghe					
Inventor's Signature						Date	03/22/01
Residence: City	Gent	State		Country	Belgium	Citizenship	BE
Post Office Address	c/o University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium						
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Residence: City	Beerse	State		Country	Belgium	Citizenship	CA
Post Office Address	c/o Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium						
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DOCKET NO. JAB-1430

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : CONTRERAS et al.

Serial No. : Art Unit:

Filed : June 4, 2001 Examiner:

For : DRUG TARGETS IN CANDIDA ALBICANS


Commissioner for Patents  
Washington, D.C. 20231ASSOCIATE POWER OF ATTORNEY

Sir:

In the matter of the above-identified application, I hereby appoint Myra H. McCormack (Reg. No. 36,602), whose postal address is One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933-7003, my associate attorney to prosecute said application, to make alterations and amendments therein, to file continuing applications claiming the benefit of said application, to receive the patent and to transact all business in the Patent Office connected with said application.

I request all communications with respect to said application be addressed to Philip S. Johnson, One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933-7003. All telephone calls should be directed to Myra H. McCormack (732) 524-6932.

Signed at New Brunswick, in the County of Middlesex and State of New Jersey, this 4th day of June, 2001.

  
Mary A. Appollina  
Reg. No. 34,087  
Attorney for Applicant(s)

One Johnson & Johnson Plaza  
New Brunswick, NJ 08933-7003  
(732) 524-3742  
DATED: June 4, 2001

## SEQUENCE LISTING

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 DeBacker, Marianne  
 Luyten, Walter  
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 10 Arg Ala Arg Gly Ile Thr Ile Ser Thr Ala His Val Glu Tyr Glu Thr  
 85 90 95  
 Lys Asn Arg His Tyr Ala His Val Asp Cys Pro Gly His Ala Asp Tyr  
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 115 120 125  
 Val Val Ala Ala Thr Asp Gly Gln Met Pro Gln Thr Arg Glu His Leu  
 130 135 140  
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 145 150 155 160  
 25 Lys Val Asp Thr Ile Asp Asp Pro Glu Met Leu Glu Leu Val Glu Met  
 165 170 175  
 Glu Met Arg Glu Leu Leu Ser Thr Tyr Gly Phe Asp Gly Asp Asn Thr  
 180 185 190  
 30 Pro Val Ile Met Gly Ser Ala Leu Met Ala Leu Glu Asp Lys Lys Pro  
 195 200 205  
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 60 Gly Tyr Lys Pro Gln Cys Phe Phe Arg Thr Asn Asp Val Thr Thr Thr  
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5 Pro Gly Asp Asn Ile Glu Met Val Gly Glu Leu Ile Lys Ser Cys Pro  
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30 Asn Ile Asn Asp Lys Thr Ile Val Gln Gly Lys Met Thr Trp Tyr Phe  
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Gly Arg Asp Pro Asn Ser Asp Leu Gln Val Ala Ser Ser Ser Arg Ile  
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35 Ser Asn Lys His Phe Gln Ile Trp Leu Asn Phe Asn Asp Lys Ser Leu  
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40 Trp Ile Lys Asp Thr Ser Thr Asn Gly Thr His Leu Asn Asn Ser Arg  
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Leu Val Lys Gly Ser Asn Tyr Leu Leu Asn Gln Gly Asp Glu Ile Ala  
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45 Val Gly Val Gly Arg Asp Glu Asp Val Val Arg Phe Val Val Val Phe  
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Gly Asp Lys Tyr Asn Pro Ala Lys Leu Pro Asp Ser Thr Asn Thr Ile  
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60 Thr Gly Gly Gly Ser Ala Met Ala Gly Val Asp Arg Glu Leu Ser Ile  
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40	Ala	Ala	Glu	Ala	Leu	Lys	His	Lys	Trp	Leu	Gln	Asp	Leu	Tyr	Glu	Glu	
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	Asp	Ser	Val	Lys	Ser	Leu	Ser	Leu	Ser	Gln	Ser	Gln	Ser	Gln	Gln	Ser	
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	Arg	Lys	Ile	Asp	Asn	Gly	Ile	His	Ile	Glu	Ser	Leu	Ser	Lys	Ile	Asp	
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	Ser	Lys	Gln	Gln	Asp	Phe	Lys	Val	Pro	Lys	Arg	Val	Ile	Pro	Leu	Ser	
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	Tyr	Gln	Ile	Asp	Pro	Arg	Thr	Asn	Lys	Lys	Val	Asp	Leu	Glu	Glu	Pro	
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	Gly	Gly	Glu	Ile	Leu	His	Leu	Phe	Phe	Asp	Pro	Leu	Ser	Ser	Gln	His	
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	Asp	Met	Ile	Met	Asp	Phe	Leu	Pro	Asn	Val	Thr	Phe	Ile	Lys	Arg	Lys	
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 Lys Thr Val Gly Arg Leu Phe Gln Ser Ile Phe Pro His Lys Pro Glu  
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 15 Leu Gln Gly Arg Gln Val Ile Thr Leu His Asn Gln Arg Asp Tyr Ile  
 225 230 235 240  
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Asn Lys Ser Arg Arg Leu Ser Met Asp Asn Gly His Cys Tyr Val Arg  
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5   Glu Ser Thr Asn Asn His His His Leu Asn Thr Val Val Asp Asn Leu  
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Arg Gln Arg Ala Gly Ser Phe Ser Phe Ile Ser His His His Asn His  
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10   His Gln Asn Ser His Asp Asn Tyr Thr Val Asp Pro Leu Thr Ser Asn  
                                   85                                  90                                  95

Gly Ala Arg Ile Ser Arg Ser Arg Ser Arg Ser Lys Ser Val Gly His  
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15   Gly Glu Ala Ile Ser Pro Ala Tyr Phe Ser Lys Asn Lys Thr Lys Asp  
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20   Leu Val Lys Gln Glu Thr Ala His Ile Ile Ser Lys Lys Leu Leu Asn  
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25   Ser Gln Gly Ser Glu Ser Lys Phe Cys Lys Ile Tyr Val Ser Asn Thr  
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Asn Asn Cys Ile Tyr Leu Pro Ala Ala Ser Ser Thr Ser Phe Thr Tyr  
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35   Glu Met Pro Thr Ala Val Asn Asn Asn Thr Leu Ser Met Asp Ser Ile  
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Asn His Ser Glu Thr Asp Phe Ser Asp Ser Pro Pro Pro Pro Asp Leu  
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40   Phe Ser Lys Met Lys Ser Phe His Ser Pro Asn Tyr Leu Thr Ser Lys  
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45   Leu Thr Lys Asp Ser Leu Ile Ile Lys Asp Leu His Phe Gln Phe Gln  
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Ser Leu Thr Thr Ile Leu Trp Pro Thr Gly Asp Ala Tyr Asn Arg Thr  
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50   His Ala Lys Glu Lys Phe Thr Ile Gly Asn Met Glu Trp Arg Thr Ser  
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55   Leu Ser Asp Ala Asp Tyr Tyr Ile Asn Ser Ser Asn Ser Asn Asp Val  
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Lys Ser Lys Asn Leu Gly Pro Glu Asp Leu Ile Asn Arg Thr Arg Glu  
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 10 Lys Pro Asp Ser Asp Asn Lys Met His His Tyr Glu Val Val Ile Asp  
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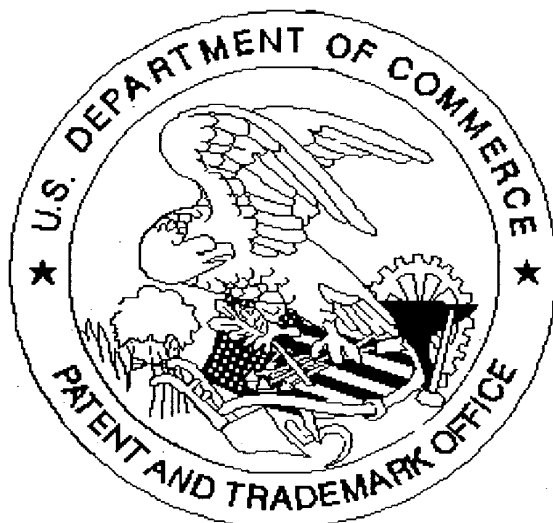
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